Enhanced Patient Serum Immunoreactivity to Recombinant Mycobacterium tuberculosis CFP32 Produced in the Yeast Pichia Pastoris Compared to *Escherichia coli* and Its Potential for Serodiagnosis of Tuberculosis

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CFP32 is a Mycobacterium tuberculosis complex-restricted secreted protein that was previously reported to be present in a majority of sputum samples from patients with active tuberculosis (TB) and to stimulate serum antibody production. CFP32 (annotated as Rv0577) was therefore considered a good candidate target antigen for the rapid serodiagnosis of TB. However, the maximal sensitivity of CFP32 serorecognition may have been limited in earlier studies because recombinant CFP32 (rCFP32) produced in *Escherichia coli* was used as the test antibody-capture antigen, a potential shortcoming stemming from differences in bacterial protein posttranslational modifications. To further investigate the serodiagnostic potential of rCFP32 synthesized in different heterologous hosts, we expressed rCFP32 in the yeast Pichia pastoris. Compared to E. coli rCFP32, yeast rCFP32 showed a higher capacity to capture polyclonal antisera in Western blot studies. Likewise, yeast rCFP32 was significantly better recognized by sera from TB patients, in enzyme-linked immunosorbent assay (ELISA), than *E. coli* rCFP32. In subsequent testing, the yeast rCFP32-based antibody-capture ELISA had a sensitivity of 85% and a specificity of 98% for the discrimination of active TB cases ($n = 40$) from BCG vaccinees ($n = 39$). The sensitivity was surprisingly high for a single-antigen TB serodiagnostic test compared to tests using *E. coli*-expressed antigens. Overall, the trans-production of rCFP32 in *P. pastoris* significantly improved the serologic detection of CFP32-specific antibodies in patient sera, thereby offering a new, possibly better, modality for producing antigens of diagnostic potential for use in the development of immunoassays for both TB and other infectious diseases (International patent request CA 2,551,537 of July 3rd, 2007).

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One year experience of APTIMA COMBO 2 Transcription Mediated Assay (TMA) for detection of Chlamydia trachomatis and *Neisseria* gonorrhoea in a large private pathology laboratory in Queensland

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Between June 2005 and July 2006, a total of 90898 samples including urine and non urine specimens were processed using the Aptima Combo 2 Assay (AC2) which has the ability to detect both Chlamydia trachomatis (CT) and Neisseria gonorrhoea (GC) in the same sample

tal specimens. CT was also detected in 69 tampon samples (12%), 34 Thin Prep samples (3.2%), 30 eye swabs (5.2%), 30 rectal swabs (8.7%), 14 throat swabs (1.4%) and 1 seminal fluid (1.1%).

Overall GC was detected in 0.7% of samples. GC was detected in 0.6% of urines specimens and 0.45% of genital swabs. GC was detected in 50 throat swabs (5.1%), 41 rectal swabs (12%), 11 tampons (1.9%) and 3 eye swabs (0.5%). Dual infections with CT and GC were detected in 0.7% of all samples. Overall prevalence of GC by culture was 0.3%. None had a positive GC culture and a negative AC2 assay result. 21% of specimens positive for GC by AC2 did not have a formal request for GC by AC2 (GCNR: GC Not Requested). 29% GCNR GC positive specimens by AC2 did not have a simultaneous culture request. 18% GCNR GC positive specimen had concurrent CT infection

Conclusions: CT and GC were detected by AC2 assay in a wide range of sample types. AC2 was more sensitive than culture for detection of GC. The ability of the assay to detect both CT and GC in one sample has emphasised the importance of testing for dual infections and enabled the un-requested GC to be detected. The data suggests that clinicians are opting for molecular- based testing for GC rather than culture- based methods.

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Application Molecular Methods on *Escherichia coli* and *Ornithobacterium rhinotracheale* Infectious in Commercial Flocks of Southern Khorasan Province in Iran

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Introduction: Ornithobacterium rhinotracheale (ORT) is a pleomorphic gram-negative Rod-shaped bacterium that has been isolated from chickens manifesting severe respiratory problems in many countries. ORT can be associated with high economic losses in poultry. Only 10 to 15% of *Escherichia coli* (*E. Coli*) intestinal coliforms are pathogenic (APEC). The aims of this study were the isolation of *Ornithobacterium rhinotracheale* and *Escherichia coli* from poultry and identification of the isolates by Polymerase chain reaction (PCR) for ORT and serology for *E. Coli*.

Materials: Samples. In this study Samples collected from lung and trachea from 13 commercially chicken flocks showing respiratory disease symptoms were pooled, homogenized and stored at -80 °C until required.

Bacteriology.

ORT: Samples were aseptically inoculated on blood agar supplemented with 7% sheep blood and 10 μg/ml gentamicin (to inhibit growth of other bacteria) to isolate and identify the causative bacteria from lung and trachea samples by routine both culture and PCR. The plates were incu-

bated in a 5—10% CO2 atmosphere at 37 °C for at least 48 hours.

E. Coli: All bacterial strains were stored in brain heart infusion broth with 20% glycerol at -80 °C prior to use. In preparation for amplification, bacterial strains were grown on either MacConkey or nutrient agar overnight at 37 °C. *E. coli* strains.

DNA extraction: Samples of The tissue followed by DNA extraction by using standard procedure according to manufacturer's instruction. Polymerase chain reaction (PCR): Methodologies for PCR analysis are described elsewhere

Results: All ORT suspicious isolates were negative in PCR. Serotypes of 13 *E. coli* isolates from flocks' colibacillosis revealed most to be O2. There were three untypable strains in the present study.

Discussion: The purpose of this study was to examine ORT and *E. coli* from chickens by culture and PCR tests. A better understanding of the virulence mechanisms of the causative APEC strains are needed to guide the development of preventive measures.

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70.029

Comparative Evaluation of Eight Methods for the Detection of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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Emergence of Methicillin-resistant Staphylococcus aureus is responsible for nosocomial and community-acquired infections worldwide. Hence, a rapid and accurate diagnosis of methicillin-resistant S. aureus in the laboratory is a vital constituent in enabling control measures and early therapeutic decisions. The present study evaluated the performance and ability of 8 different methods for the identification of methicillin-resistant S. aureus. A total of 207 S. aureus clinical isolates consisting of 89 MRSA strains (oxacillin MIC $\geq$ 8 mg/L), 118 methicillin-susceptible S. aureus (oxacillin MIC $\leq$ 4 mg/L) were included in the study. The S. aureus strains were further confirmed by Staphylase-latex agglutination and tube coagulase tests. MRSA strains were evaluated by six different susceptibility testing methods namely, Chromogenic MRSA agar (CMRSA), Oxacillin resistance screen agar base (ORSAB), Kirby-Bauer disc diffusion test using 1 μg oxacillin disc (MHA-O), Mannitol salt oxacillin agar (MSO) and Mannitol salt cefoxitin agar with two different concentrations of cefoxitin [4 mg/L (MSC-4) and 6 mg/L (MSC-6)]. MRSA strains were further evaluated with two additional methods, namely, MRSA-screen latex agglutination test and mecA PCR. All these 8 tests results were compared to oxacillin E-test as gold standard. The mecA PCR and MRSA-screen latex tests results showed 100% sensitivity, specificity, PPV and NPV. After 24 h of incubation on CMRSA, ORSAB, MSO, MSC-4 and MSC-6 medium, 96.6%, 97.8%, 94.4%, 100% and 97.8% of the MRSA strains, respectively, were detected. The sensitivities and specificities of the six different media are the following, CMRSA (96.6% and 95.8%), ORSAB (97.8% and 96.6%), MSO (97.5% and 96.6%), MSC-4 (100% and 83.1%), MSC-6 (97.8% and 94.9%) and MHA-O (98.3% and 97.8%). It was found that MSO was the inexpensive test ($0.70) while MRSA latex was the most expensive method ($4.00). In conclusion, mecA PCR is the most accurate, reliable and low-priced ($1.60) method for the detection of MRSA from clinical samples.

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Viral Diagnostics (Poster Presentation)

71.001

Detection of the Rate and Genotyping of Hepatitis C Virus (HCV) Infection in Haemophilia and Thalassemia Patients in Iran

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Background: Hepatitis C is one of the blood transmitted infectious diseases. The virus belongs to flaviviridae and has 6 major genotypes. Haemophilia and thalassemia patients, dialysis and drug injecting patients are at high risk of acquiring hepatitis C virus infection.

Methods: The rates of HCV infection were detected among 103 anti-HCV positive haemophilia and thalassemia patients in Isfahan province (Iran) by nested PCR. Then the genotypes of the isolated viruses detected by Reverse hybridization (Lipa) method

Results: 41% of the total 103 samples showed negative PCR results and 59% of them had positive PCR results. The rates of HCV infection was 60% in hemophiliacs and 58% in thalasemics. Also genotyping analysis in hemophiliacs and thalassemics detected type 1 in 59%, type 3a in 29% and type 6a in 8% of samples studied.

Conclusion: In the present study the predominant types of HCV in the patients were type 1 and 3a which is similar to results obtained from investigations in the other areas in Iran, but we detected mixed types in some patients and the type 6a in one patient. The type 6a is not a common type in Iran and not seen in previous studies. The origin of this type which detected in a hemophilia patient, may be from foreign concentrated clotting factors at the times in which the factors were not decontaminated with heating processes.

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